ANCA-associated glomerulonephritis : insights into etiology, pathogenesis, and prognosis

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Education's purpose is to replace an empty mind with an open one.
Malcolm Forbes

Chapter 7

General discussion

Anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis is a potentially life-threatening systemic disease. Renal involvement is an unfavorable factor in terms of prognosis and outcome. ANCA-associated renal disease can be treated effectively with immunosuppressive drugs, but administration of these drugs is often not without side-effects. Since these drugs can cause morbidity and mortality, they need to be used with care. For each patient a risk-benefit calculation should be made. Patients should not be exposed to the risks of treatment if improvement in outcome is not likely. Besides renal damage, for therapeutic decisions the involvement of other organs should be taken into account. In this thesis, outcome parameters are investigated in depth, providing more insight into which variables are favorable and unfavorable with regard to treatment response. Both short and long term outcome for patients who present with severe renal impairment were topics of investigation. Long term outcome was also studied for patients with mild to moderated renal involvement. The patients that were dialysis dependent at diagnosis and those who had ENT involvement were analyzed separately. This thesis attempts to elucidate more of the aspects of renal histology in ANCA-associated vasculitis and its relation to clinical outcome.

Nature versus nurture

Which genetic and/or environmental factors contribute to or are necessary for the initiation of ANCA-associated vasculitis has been subject of debate for some time. Reviewing the literature, the most likely hypothesis is that an interplay of environmental factors upon genetic susceptibility is responsible for the eventual development of clinical disease. In chapter 6, different factors possibly involved in the development of ANCA are extensively reviewed. Of all the candidate factors to play a role in the etiology of ANCA-associated vasculitis, many did not turn out to be closely related to, let alone necessary for, the development of vasculitis, later on. From the course of research within this field we can conclude that not a single factor has been identified that is absolutely necessary for the development of ANCA-associated vasculitis. The hypothesis would be that vasculitis is a multifactorial disease initiated by both environmental and genetic factors.

The most likely candidate environmental factors that trigger ANCA production are silica exposure, bacterial infection, e.g. by *S. aureus*, viral infection, e.g. by parvovirus B19, and drugs like anti-thyroid medication or antibiotics. The identification of geographical and seasonal trends also points towards the

presence of an environmental initiating factor. Alternatively, many different genes have been identified to be associated with the presence of ANCA-associated vasculitic disease. These genes regulate for example HLA, apoptosis, Fcγ-receptor, complement, adhesion of polymorphonuclear cells, T cells, cytokines, PR3 inhibitor, PR3, and MPO.
The hypothesis that vasculitis is triggered by a multifactorial interplay is supported by the low incidence of ANCA-vasculitis, possibly reflecting the chance of these factors coming together and inducing disease. Another characteristic of the disease supporting this hypothesis is the older age of onset, indicating that on average a long time is necessary to gather the factors that are necessary for disease initiation. Older age of onset also points towards the importance of an environmental factor, rather than a genetic factor alone.

Predicting renal outcome over time

Prognostic considerations in ANCA-associated vasculitis of course regard survival, but also persistent organ damage due to necrosis and fibrosis after active vasculitis and/or granuloma formation. The impact of end-stage renal disease, with poor quality of life and increased morbidity and mortality, justifies separate consideration with respect to prognosis in ANCA-vasculitis.
From the consultant physician's point of view, renal prognosis is a challenging issue, especially when patients present with acute and severe renal involvement upon diagnosis. Treatment of these patients requires strong immunosuppressive therapeutic regimens. Drawbacks of these regimens are severe and potentially lethal adverse effects [1]. Renal function is sometimes insufficiently restored [2], and 90% of patients experience persistent morbidity despite adequate treatment [3].
This thesis considers several aspects regarding prognosis: two studies exclusively focus on which histological and clinical determinants are important for predicting renal and patient outcome after one year and after 5 years (chapter 2 and 4), another study considers the hazards and benefits of treatment for patients with severe ANCA-associated glomerulonephritis (chapter 3).
The studies on prediction in this thesis (chapters 2, 3, and 4) should be interpreted within the limits of the patient group the predictive models were constructed for. In our analyses, patients with newly diagnosed mild to moderate or severe renal disease who had no previous history of extrarenal vasculitis were included. These trials did not include patients who experienced a second renal flare or multiple renal flares. Their prognosis would undoubtedly be worse due to previous organ damage. Therefore, extrapolation of the results to patients not meeting the inclusion criteria of the trials is not advisable.

Another point of consideration is that the r-values of the univariate analyses could raise some doubt on the clinical applicability of the findings. A possible explanation for the relatively low r-values could be that the patient cohorts are a predefined group of patients with poor or mild to moderate renal function at entry, with parameters in a relatively tight range, which is bound to lead to relatively low r-values. Therefore, it was necessary not only to analyze the data in a univariate manner, but also in a multivariate manner, since the combination of parameters predicts much better for outcome than single parameters alone.

The models obtained from regression analysis can be useful to the clinician for estimating renal status after 12 months. The models resulting from the different studies described in this thesis are listed in Table 1. Despite the limited predictive values of the models (r^2 for GFR_{12} = 0.491 and r^2 for dialysis at 12 months = 0.309) described in chapter 2, these models clearly showed that consideration of several clinical and histological parameters results in a better prediction of renal outcome after 12 months than evaluation of GFR_0 (r^2 for GFR_{12} = 0.084 and r^2 for dialysis at 12 months = 0.073) or dialysis dependency at entry (r^2 for GFR_{12} = 0.075 and r^2 for dialysis at 12 months = 0.091) alone. Even at 5 years, the combination of clinical and histological parameters predicted fairly well for outcome (r^2 for GFR_5 = 0.574 and r^2 for ESRF = 0.389 for patients with mild to moderate renal involvement and r^2 for GFR_5 = 0.307 and r^2 for ESRF = 0.389 for patients with severe renal disease), as described in chapter 4.

Table 1. Predictors of short and long term outcome

Patient group	Outcome	Short term predictors	Long term predictors
Patients with	Recovery	-	GFR0, tubular atrophy
serum creatinine	ESRF	-	Age, tubular necrosis
200-500 µmol/L	GFR	GFR0, fibrinoid necrosis, segmental crescents	GFR0, age, fibrinoid necrosis, glomerulosclerosis
	Alive	-	None
Patients with	Recovery		Large-vessel sclerosis, arteriosclerosis
serum creatinine	Dialysis/ESRF	Adjunctive treatment, normal glomeruli	Adjunctive treatment, fibrous crescents, tubular necrosis
>500 µmol/L	GFR	GFR0, normal glomeruli, tubular atrophy, intraepithelial infiltrate, age	Age
	Alive	None	Age

Short term predictors for patients with serum creatinine 200-500 µmol/L was at 18 months after diagnosis and for patients with serum creatinine >500 µmol/L at 12 months after diagnosis. Long term outcome for both groups was evaluated at 5 years after diagnosis. Adjunctive treatment was either intravenous methyl prednisolone or plasma exchange.

The combination of normal glomeruli, acute and chronic tubulo-interstitial damage, age, and treatment was predictive of renal outcome at 12 months (chapter 2). A worse outcome in patients with a low percentage of normal glomeruli, more acute and chronic tubulo-interstitial lesions, and intravenous methyl prednisolone as adjunctive treatment was observed. A greater extent of acute and chronic glomerular and interstitial lesions predicted a worse renal function and higher chance of dialysis dependency at entry. After calculation

of GFR_{12} corrected for GFR_0, age, the percentage of normal glomeruli, tubular atrophy, and intra-epithelial infiltrates proved to be important predictors of renal function recovery and also to be independent of renal function at entry.
In patients who were dialysis dependent at entry, factors determining outcome were also studied (chapter 3). Variability in outcome was determined by: type of adjunctive treatment, percentage of normal glomeruli and glomerulosclerosis, the extent of tubular atrophy, and the presence of arteriosclerosis. Of note is that in both this population and the whole population of patients with a serum creatinine of over 500 µmol/L, the percentage of normal glomeruli and the extent of tubular atrophy are important determinants of outcome.
Predictive parameters of long term outcome were analyzed in chapter 4. Our data show that in mild to moderate ANCA-associated glomerulonephritis, renal function at diagnosis, age, and acute and chronic tubular injury are predictive of renal outcome at 5 years. Meanwhile, in patients with severe renal disease, adjunctive therapy, acute tubular lesions, and chronic glomerular and vascular lesions determined renal outcome at 5 years.
It is remarkable that outcomes after 1 and 5 years in patients with severe renal disease at diagnosis share the same predictors: acute tubular injury and the type of adjunctive treatment (administered within the first two weeks after diagnosis). Renal outcome after 5 years was heavily determined by renal outcome after 1 year. There seems to be a selection within the first year after diagnosis. If a patient survives the first year, the chances of surviving 5 years are 78% for severe renal disease and even 90% for mild to moderate renal disease. Meanwhile, of the patients who were on dialysis at 1 year, about half will die within the next 4 years and the rest will remain in ESRF.

The role of unaffected glomeruli in the course of ANCA-associated glomerulonephritis

One striking feature that often results from clinico-histopathological analyses is the relationship between unaffected glomeruli and favourable outcome. In several studies, normal glomeruli have been described to be associated with and even predict for renal outcome over time in terms of GFR or serum creatinine [4-10]. Other studies have either not studied this association [11], or not found this, possibly due to the limited number of patients analyzed [12]. The fraction of normal glomeruli has even been directly related to renal function. An obvious explanation for this relationship would be that when more normal glomeruli are present, less glomeruli contain active or chronic lesions. However,

in most studies, active and chronic lesions as such were not predictors for renal function, and if they were, normal glomeruli had superior predictive value. One reason for this might be that active lesions over time can either return into normal glomeruli or develop into chronically damaged ones. Active lesions are reversible until a certain point in time [13]. In an earlier EUVAS study on biopsies from patients with mild to moderate renal involvement from the CYCAZARAM trial, more active lesions, like fibrinoid necrosis and segmental crescents, were predictive of better renal outcome, implying a capacity of these lesions to recover [5]. In a similar study described in this thesis (chapter 2 and 4) in patients with severe renal involvement, active glomerular lesions had no predictive value neither for short or long term, implying that in these patients a larger amount of glomeruli containing active lesions had lost their regenerative capacity [14].

Another reason for the superior reliability of normal glomeruli as predictive parameter for renal outcome might be that -under treatment- the percentage of normal glomeruli in a renal biopsy does not change over time [15]. In contrast, the percentage of crescents decreases, while the percentage of glomerulosclerosis increases, usually resulting in poor correlation of these parameters with prognosis.

The initiation of treatment has been suggested to prevent crescent formation in as yet unaffected glomeruli, while it has been shown to be unable to prevent glomerulosclerosis of affected glomeruli [16]. In a rabbit model, crescents, present in 90% of glomeruli at onset, can evolve into histologically normal glomeruli in half of the cases, and in glomerulosclerosis in the other half [17]. In humans, spontaneous improvement in crescentic glomerulonephritis has also been described [18,19]. The hypothesis derived from this would implicate a point-of-no-return, after which glomeruli are too badly damaged by crescent formation to reverse into normal again, and instead evolve to sclerosis. This point could be when crescents turn fibrous [18]. After induction of remission, normal glomeruli are not recruited in the active disease process anymore, reflected by stabilisation of renal function. Only during relapse of the disease, normal glomeruli can again become damaged and may develop extracapillary proliferation and glomerulosclerosis [15].

Tubulitis and pathogenesis

The significance of renal tubulointerstitial damage in Wegener's granulomatosis in terms of outcome has been appreciated before [20]. Nonetheless, glomerular lesions [21] (although sometimes in combination with interstitial lesions [22,23]) have usually been the subject of outcome studies.

In multiple forms of primary glomerulonephritis that were not ANCA-associated, tubulointerstitial lesions have been found to correlate with long term patient and renal survival [24,25]. Tubular atrophic changes and interstitial fibrosis were predominantly present in crescentic, focal sclerosing, chronic diffuse, and membranoproliferative glomerulonephritis [25].

In chapter 2, we describe that tubular damage, especially tubular atrophy and intra-epithelial infiltrates in biopsies from patients who initially present with a serum creatinine higher than 500 μmol/L, were even more predictive of renal outcome than glomerular lesions [14].

However, the percentages of different infiltrating cells have merely been correlated and have not yet been co-localized with tubular lesions. Although granulocyte, monocyte/macrophage, and lymphocyte infiltration in the interstitium has been described [26], the extent of these infiltrates in tubuli is still to be explored. However, circumstantial evidence of monocyte recruitment exists. Renal tubular cells express proteins associated with interstitial monocyte infiltration [27], cell-directed adhesion and chemotaxis [26]. IL1-β, ICAM-1 and VCAM-1 were found in tubular areas of biopsies and are involved in the recruitment of intraglomerular leukocytes in renal vasculitis [28]. Cytokines expressed by mononuclear cells were also observed in some tubular epithelial cells [29,30]. Moreover, renal tubular epithelial cells have been shown to express both PR3 mRNA [31] and protein [32,33], although the latter has been disputed [34]. Antibodies to PR3 have been shown to interact with tubular epithelial cells that express PR3 [33,35], implying that anti-PR3 antibodies can directly cause renal damage and loss of kidney function in WG and not only secondary to glomerular injury.

Dying of therapy

In an analysis on causes of death within patients on dialysis at entry (chapter 3), an important finding was the distribution of time to death in relation to its causes. A subdivision emerged between the first 3 months of disease and the period after 3 months. In the first 3-month period, death most often resulted

from causes that were not clearly therapy related, such as the disease itself and vascular factors. Between 3 and 12 months, death was exclusively caused by therapy, predominantly sepsis following infection. Considering earlier reports that the addition of plasma exchange and intravenous methyl prednisolone facilitates the improvement of renal function within the first 3 months after disease onset [36-38], it is a striking contrast that death due to therapy almost exclusively occurs in the period after 3 months. The explanation of this phenomenon may be the cumulative dose of immunosuppressive treatment. This finding stresses once again that improved and safer treatment regimens are required for patients with ANCA-associated glomerulonephritis and dialysis dependency at diagnosis.

The protective effect of ENT involvement

In many patients diagnosed with ANCA-associated vasculitis renal biopsies show that both acute and chronic lesions are already present at diagnosis. A previous study has shown that under treatment no normal glomeruli will participate in the disease process [15]. Therefore, a major challenge lies in the early recognition of ANCA-associated vasculitis. This thesis shows that patients with ENT involvement have a higher percentage of normal glomeruli and less chronic glomerular and tubular damage in their biopsies (chapter 5). These parameters have been shown to be beneficial in terms of outcome over time (chapters 2, 3, and 4). ENT involvement has even been shown to predict longer survival in systemic Wegener's granulomatosis [39]. Next to ENT involvement, general symptoms are often present at diagnosis, like myalgia, arthralgia/ arthritis, body temperature over 38 °C, and weight loss ≥2 kg. These general complaints should be noticed since they can reduce diagnostic delay (chapter 5).

Conclusions

This thesis elaborates on the different factors that might play a role in ANCA formation in patients with ANCA-associated vasculitis. It reveals which clinical and histological parameters determine outcome over time, differentiating between groups of patients. These efforts are aimed at rendering insight into the prognoses of different patient groups and the influence of the clinical picture and the renal biopsy of the individual patient on the prognosis.

The European Vasculitis Study Group (EUVAS)

All the work presented in this thesis has been the result of a long tradition of intense and constructive collaboration within the EUVAS. The only way to gain knowledge on rare diseases, like ANCA-associated vasculitis, is to work within a large nation-transcending consortium. Over the past years, the EUVAS has contributed significantly to the existing knowledge on ANCA-associated vasculitis, especially when it comes to therapeutic strategies. International large-scale collaboration, as established in the EUVAS, will play a pivotal role to study more aspects of ANCA-associated vasculitis in the future.

Future perspectives

Over the past two decades we have learned a lot about the pathogenesis of vasculitis, but also about response to treatment and about which factors determine prognosis. Future research will probably render insight into which genetic and environmental factors are important in developing disease and will provide specific population-based or even individual-based therapeutic regimens. Considering histology with regard to ANCA-associated glomerulonephritis, it not only has a role in confirming diagnosis, but also has prognostic potential -both at short and long term. Histology can help to provide insight into the pathogenesis of this disease. Due to recent observations from both our group and others, the role of intra-epithelial infiltrate within the tubules becomes of special interest, and might provide a challenging future field of research.

However, the greatest challenge in the future will be to find answers to basic questions the newly diagnosed vasculitis patient asks his treating physician, like: "How did I get this disease?", "How can I be cured from it?", and "What is my prognosis?". These questions touch upon fundamental issues when it comes to insight into etiology and pathogenesis of vasculitis. But also the search for a treatment that cures the disease rather than one that only manages its symptoms remains a future challenge.

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